HIV-Inhibitory and Cytotoxic Oligostilbenes from the Leaves of *Hopea malibato*[1]

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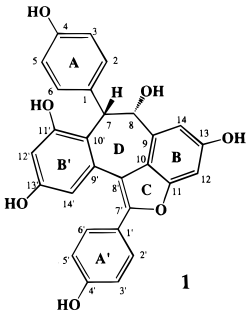
Three new oligostilbenes, malibatols A (**1**) and B (**2**) and dibalanocarpol (**3**), together with one known oligostilbene balanocarpol (**4**), were isolated from the organic extract of the leaves of *Hopea malibato*. The structure elucidation of these compounds was based on the interpretation of their chemical and spectral data. Compounds **3** and **4** exhibited very modest HIV-inhibitory activity, while compounds **1** and **2** were cytotoxic to the host cells (CEM SS) in the antiviral assay.

Several oligostilbenes and triterpenes have previously been isolated from species of genus *Hopea*.[2−7] In our continuing screening of natural products for HIV-inhibitory agents,[8] we have isolated three new oligostilbenes, malibatols A (**1**) and B (**2**) and dibalanocarpol (**3**), along with a known oligostilbene, balanocarpol (**4**), from the organic extract of the leaves of *Hopea malibato* Foxw. (Dipterocarpaceae). All these compounds are closely related; their structures were elucidated on the basis of the interpretation of their chemical and spectral data.



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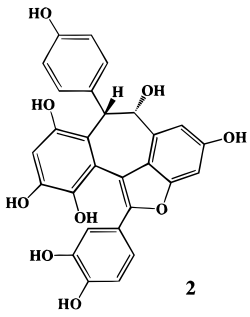

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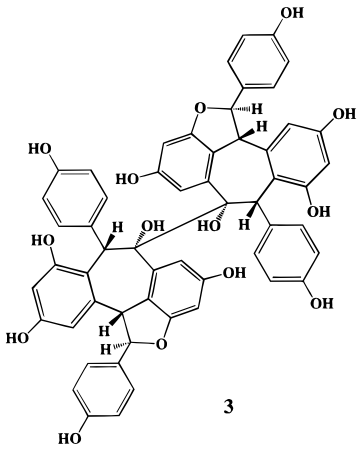

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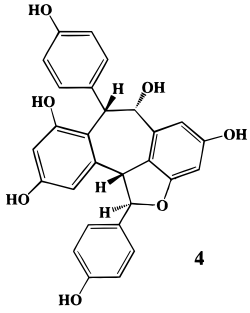

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Results and Discussion

The molecular formula of malibatol A (**1**) was established by HRFABMS as $C_{28}H_{20}O_7$, indicating 19 degrees of unsaturation. The ^{1}H NMR spectrum of **1** consisted of five pairs of peaks. Two pairs appeared at δ 7.02/6.33 and 7.45/6.80, each with characteristic *ortho* and *meta* couplings; each peak integrated as two protons. They were assigned to the protons of two *para*-disubstituted aromatic rings, A and A′. Two other pairs resonating at δ 7.01/6.57 and 6.51/6.30 were assigned as *meta* protons on two tetrasubstituted aromatic rings, B and B′. The remaining pair of proton resonances appeared at higher field, δ 5.46 and 5.28; their coupling constant (2.5 Hz) suggested that they were alicyclic vicinal protons in a *cis* configuration. In a COSY experiment, long-range couplings between signals at δ 5.46 and 7.02, as well as 5.28 and 7.01, indicated that the two higher field protons were benzylic. The ^{13}C NMR spectrum of **1** showed signals for 28 carbons, only two of which could be attributed to aliphatic (sp^3) carbons (δ 48.8 and 74.8), including one secondary alcoholic carbon. Six signals appearing between δ 159.1 and 155.2 ppm were regarded as phenolic carbons. The 12 signals between δ 130.9 and 95.9 ppm represented the protonated aromatic carbons. The remaining signals were attributed to eight quaternary carbons and confirmed by a DEPT experiment. Therefore, compound **1** consisted of four aromatic rings (A, A′, B, and B′) with five phenolic hydroxy groups (IR, 3326, 1611, and 1511 cm^{-1}). This left two sp^2 and two sp^3 carbons and three sites of unsaturation unaccounted for; therefore, there were two additional rings in the compound. Structure **1** was completed by analysis of HMBC data. As illustrated in Figure 1, key HMBC correlations between protons adjacent to ring connection points and carbons on the opposite ring allowed placement of the remaining carbons and assembly of the hexacyclic structure. The NOE enhancement between H-7 and H-8 observed in an NOE experiment confirmed their *cis* configuration in **1** (Figure 2).

Malibatol B, **2**, exhibited IR, 1D- and 2D-NMR spectral data similar to those of compound **1**, which suggested that compound **2** was another new, structurally related oligostilbene. Its HRFABMS provided the

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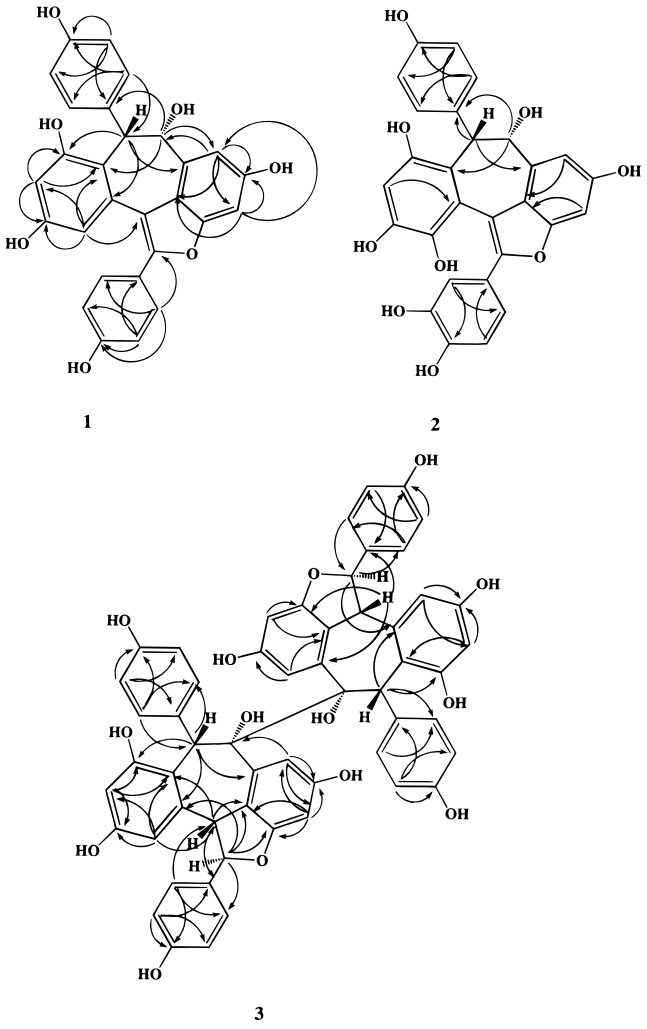

Figure 1. Key HMBC correlations of compounds **1**−**3**. Arrows indicate correlation between hydrogen (point of origin) and carbon (arrowhead).

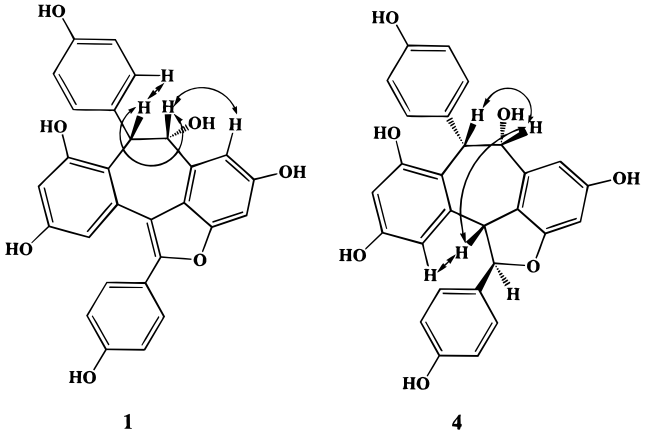

Figure 2. Key NOE interactions of compounds **1** and **4**.

molecular formula of $C_{28}H_{20}O_9$, two more oxygens than malibatol A (**1**). The ^{1}H NMR spectrum of **2** exhibited one aromatic proton less in both of the rings A′ and B′ than compound **1**, which indicated that an aromatic proton in each of those rings had been replaced by a hydroxyl group. The ^{13}C NMR spectrum of **2** confirmed the hydroxyl substitutions at C-3′ (δ 159.3) and C-14′ (δ 158.7). This explanation was fully supported by HMQC and HMBC experiments. Thus, malibatol B (**2**) was identified as 3′,14′-dihydroxymalibatol A.

Compound **4** was structurally related to **1**, except that the furan ring (C) was partially reduced. It was identified as balanocarpol, previously isolated from *Balanocarpum zeylanicus* and *Hopea jucunda*.[2] On the other hand, compound **3** exhibited complex ^{1}H and ^{13}C NMR spectra that resembled those of **4**; its molecular formula was established as $C_{56}H_{42}O_{14}$ (by HRFABMS), equivalent to two molecules of **4** less two hydrogens. Since there were no proton signals corresponding to the two C-8 positions in the ^{1}H NMR spectrum, it appeared that two molecules of **4** were connected at their C-8 positions to form **3**. HMBC experiments confirmed the structures of the two halves and identified the two C-8 carbons as tertiary alcohols and the linkage points for the monomeric halves. The relative configurations at C-7 and C-8′ were assigned by analogy with **4**, but the configuration at C-8 could not be determined.

Compounds **1**−**4** were tested in vitro in the NCI primary anti-HIV screen;[8,9] compounds **3** and **4** exhibited very modest HIV-inhibitory activity (EC_{50} values of 46 and 20 μg/mL, respectively), while compounds **1** and **2** showed only cytotoxicity to the host cells (CEM-SS; IC_{50} values of 13 and 21 μg/mL, respectively).

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter in CH_3OH. UV spectra were recorded on a Beckman DU-64 spectrophotometer. FT-IR spectra were obtained on a Perkin-Elmer 267 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian VXR-500 spectrometer using CD_3OD as solvent and internal standard. The number of attached protons for each carbon was determined from DEPT experiments. HPLC was performed on a Waters 600E system equipped with a Water 990 photodiode array detector.

Collection and Extraction. Leaves of *H. malibato* Foxw. (Dipterocarpaceae) were collected in Luzon, Philippines, on April 3, 1987, by J. S. Burley and D. D. Soejarto. A voucher specimen has been deposited in the Field Museum, Chicago, IL. The dried leaves were ground (695 g) and then percolated at room temperature in MeOH−CH_2Cl_2 (1:1) followed by 100% MeOH. Solvents from the combined organic extracts were removed in vacuo to provide a total of 93.32 g (13% dry weight) of crude organic extract.

Isolation. A 9.70 g portion of the organic extract was subjected to a solvent/solvent partitioning protocol to yield hexane (1.01 g), CCl_4 (0.50 g), $CHCl_3$ (1.20 g), EtOAc (4.08 g), and H_2O (2.81 g) fractions. The HIV-inhibitory EtOAc fraction was permeated (200 mg × 4) through a Sephadex LH-20 column (2.5 × 85 cm) with MeOH; seven fractions were obtained. Portions of the HIV-inhibitory fractions F (44 mg) and G (48 mg) were subjected to HPLC on a cyano-bonded phase column (1 × 25 cm); elution with hexane−*i*-PrOH (3:2) provided compounds **1** (4.6 mg, 0.25% yield from the crude extract), **2** (2.1 mg, 0.12% yield), **3** (21.5 mg, 1.2% yield), and **4** (7.3 mg, 0.40% yield), as off-white or tan solids.

Malibatol A (1): $[\alpha]_D$ −38° (*c* 0.37, CH_3OH); UV (MeOH) λ_{max} (log ϵ) 204 (4.6), 251 (4.3), 305 (4.0), 312 (4.0), 332 (4.1) nm; IR (film) ν_{max} 3326, 1611, 1511, 1436,

1237, 1139, 1014, 832 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 7.45 (2H, dd, J = 8.5, 2.5 Hz, H-2′, 6′), 7.02 (2H, dd, J = 9.0, 2.5 Hz, H-2, -6), 7.01 (1H, dd, J = 2.0, 1.0 Hz, H-14), 6.80 (2H, dd, J = 8.5, 2.5 Hz, H-3′, -5′), 6.57 (1H, dd, J = 2.0, 1.0 Hz, H-12), 6.51 (1H, d, J = 2.5 Hz, H-14′), 6.33 (2H, dd, J = 9.0, 2.5 Hz, H-3, -5), 6.30 (1H, d, J = 2.5 Hz, H-12′), 5.46 (1H, dd, J = 2.5, 1.0 Hz, H-7), 5.28 (1H, ddd, J = 2.5, 1.0, 1.0 Hz, H-8); ^{13}C NMR (125 MHz, CD_3OD) δ 159.1 (C-4′), 157.5 (C-11′), 156.7 (C-13′), 156.2 (C-11), 155.4 (C-13), 155.2 (C-4), 151.2 (C-7′), 139.7 (C-9), 135.8 (C-9′), 133.4 (C-1), 130.9 (C-2′, -6′), 130.6 (C-2, -6), 124.7 (C-1′), 121.3 (C-10′), 119.1 (C-10), 117.3 (C-8′), 116.4 (C-3′, -5′), 114.7 (C-3, -5), 109.9 (C-14), 109.7 (C-14′), 102.2 (C-12′), 95.9 (C-12), 74.8 (C-8), 48.8 (C-7); LRFABMS m/z 468 [M^+] (5), 451 (7), 433 (4), 386 (50), 371 (30), 329 (45), 307 (100), 289 (64), 279 (32), 275 (40), 220 (38); HRFABMS m/z 468.1215, calcd for $C_{28}H_{20}O_7$, 468.1209.

Malibatol B (2): $[\alpha]_D$ −77° (c 0.06, CH_3OH); UV (MeOH) λ_{max} (log ϵ) 214 (4.5), 223 (4.5), 227 (4.5), 265 (4.5), 276 (4.5) Hz; IR (film) ν_{max} 3354, 2359, 1539, 1506, 1456, 1362, 1127, 668 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 9.34 (1H, d, J = 2.0 Hz, H-2′), 8.17 (1H, d, J = 9.0 Hz, H-5′), 7.12 (1H, dd, J = 9.0, 2.0 Hz, H-6′), 6.96 (1H, dd, J = 2.0, 1.0 Hz, H-14), 6.86 (1H, d, J = 2.0 Hz, H-12), 6.77 (2H, dd, J = 9.0, 2.0 Hz, H-2, -6), 6.74 (1H, s, H-12′), 6.27 (2H, dd, J = 9.0, 2.0 Hz, H-3, -5), 5.77 (1H, d, J = 3.0 Hz, H-7), 5.46 (1H, dd, J = 3.0, 1.0 Hz, H-8); ^{13}C NMR (125 MHz, CD_3OD) δ 168.2 (C-11), 159.3 (C-3′), 158.7 (C-14′), 158.2 (C-11′), 157.2 (C-13′), 156.7 (C-4′), 155.7 (C-13), 155.4 (C-4), 152.0 (C-7′), 139.3 (C-9), 137.6 (C-9′), 133.9 (C-1), 132.4 (C-1′), 131.2 (C-2, -6), 122.0 (C-5′, -10′), 116.2 (C-10), 115.9 (C-8′), 115.6 (C-6′), 114.8 (C-3, -5), 114.6 (C-2′), 109.2 (C-14), 102.0 (C-12′), 96.6 (C-12), 75.0 (C-8), 49.3 (C-7); LRFABMS m/z 501 [MH]$^+$ (1), 500 (1), 487 (1), 468 (2), 442 (3), 413 (4), 329 (24), 257 (3), 234 (5), 177 (18), 176 (100); HRFABMS m/z 501.1186, calcd for $C_{28}H_{21}O_9$ 501.1188.

Dibalanocarpol (3): $[\alpha]_D$ −227° (c 0.73, CH_3OH); UV (MeOH) λ_{max} (log ϵ) 378 (3.4), 346 (3.6), 286 (4.4), 221 (5.2), 216 (5.2) nm; IR (film) ν_{max} 3362, 2358, 1616, 1558, 1516, 1456, 1362, 1232, 1171, 1125, 1006, 835, 668 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 6.88 (2H, dd, J = 8.5, 2.5 Hz, H-2‴, -6‴), 6.86 (2H, dd, J = 8.5, 2.5 Hz, H-2, -6), 6.76 (2H, dd, J = 8.5, 2.5 Hz, H-2′, -6′), 6.71 (1H, dd, J = 8.5, 2.5 Hz, H-5′), 6.70 (1H, dd, J = 8.5, 2.5 Hz, H-3′), 6.64 (1H, dd, J = 8.5, 2.5 Hz, H-5‴), 6.62 (1H, dd, J = 8.5, 2.5 Hz, H-3‴), 6.51 (2H, dd, J = 8.5, 2.5 Hz, H-2″, -6″), 6.48 (1H, d, J = 2.5 Hz, H-12′), 6.40 (2H, dd, J = 8.5, 2.5 Hz, H-3″, -5″), 6.38 (2H, dd, J = 8.5, 2.5 Hz, H-3, -5), 6.24 (1H, d, J = 2.5 Hz, H-14), 6.20 (1H, d, J = 2.5 Hz, H-12), 6.19 (1H, d, J = 2.5 Hz, H-12″), 6.12 (1H, d, J = 2.5 Hz, H-14‴), 6.10 (2H, d, J = 2.5 Hz, H-14′, -14″), 5.78 (1H, d, J = 2.5 Hz, H-12‴), 5.10 (1H, d, J = 2.5 Hz, H-7‴), 4.46 (2H, d, J = 2.5 Hz, H-7, -7″), 4.44 (1H, d, J = 2.5 Hz, H-7′), 3.67 (2H, d, J = 3.0 Hz, H-8′, 8‴); ^{13}C NMR (125 MHz, CD_3OD) δ 163.9 (C-11″), 161.3 (C-11), 160.4 (C-11′), 159.4 (C-13‴), 159.0 (C-13″), 158.9 (C-4′), 158.4 (C-13), 158.3 (C-4″), 158.2 (C-11‴), 158.0 (C-13′), 157.9 (C-4‴), 155.5 (C-4), 148.6 (C-9″), 142.9 (C-9), 140.0 (C-9′), 136.1 (C-9‴), 134.8 (C-1‴), 134.3 (C-1), 133.7 (C-1″), 132.6 (C-1′), 131.0 (C-2, -6), 129.6 (C-2‴, -6‴), 129.5 (C-2′, -6′), 127.2 (C-2″, -6″), 122.7 (C-10‴), 120.3 (C-10′), 120.1 (C-10″), 118.5 (C-10), 116.8 (C-5′), 116.4 (C-5‴), 116.3 (C-3′), 116.1 (C-3‴), 115.6 (C-3″, -5″), 114.5 (C-5), 113.1 (C-3), 105.3 (C-14), 101.9 (C-14′), 97.6 (C-14″), 97.2 (C-14‴), 96.5 (C-12), 95.7 (C-12‴), 95.2 (C-12′), 94.7 (C-12″), 94.5 (C-7‴), 91.1 (C-7′), 75.2 (C-8, -8″), 58.2 (C-8‴), 57.5 (C-8′), 53.1 (C-7), 52.5 (C-7″); LRFABMS m/z 939 [MH]$^+$ (1), 938 [M]$^+$ (1), 905 (1), 684 (1), 640 (1), 596 (1), 552 (1), 508 (1), 176 (92), 154 (100); HRFABMS m/z 939.2655, calcd for $C_{56}H_{43}O_{14}$ 939.2653.

Balanocarpol (4): $[\alpha]_D$ −18° (c 0.52, CH_3OH); HR-FABMS m/z 471.1445, calcd for $C_{28}H_{23}O_7$ 471.1446; all spectral data are in accord with literature reports.[2]

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